

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Claim 1. (currently amended)** A method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule, said method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, a nucleic acid polymerase and a target nucleic acid, wherein the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal to form a labeled primer, the nucleic acid amplification reaction being performed until the primer present in a lower number is consumed;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected; and

(d) quantifying the target nucleic acid on the basis of evaluation results.

**Claim 2. (previously presented)** A method according to claim 1, wherein the measurement step includes a step of measuring an amount of the marker molecule present in a predetermined micro detection field, said marker molecule being contained in the labeled primer attached to the target nucleic acid.

**Claim 3. (previously presented).** A method according to claim 2, wherein, in the measurement step, the measurement is performed in a fluid.

**Claim 4. (previously presented)** A method according to claim 3, wherein the evaluation step comprises a measurement which is affected by fluorescence correlation spectroscopy.

**Claim 5. (canceled)**

**Claim 6. (canceled)**

**Claim 7. (previously presented)** A method according to any one of claims 1 to 5, wherein the quantifying of the target nucleic acid includes determining the presence and absence of the marker molecule of the labeled primer attached to the target nucleic acid and incorporated into products of the nucleic acid amplification reaction on the basis of the evaluation results.

**Claim 8. (previously presented)** The method according to any one of claims 1 to 5, wherein the quantifying of the target nucleic acid includes determining the number of the labeled primer attached to the target nucleic acid and incorporated into products of the nucleic acid amplification reaction on the basis of the evaluation results.

**Claim 9. (withdrawn)** An apparatus of analyzing a target nucleic acid by applying a nucleic acid amplification reaction

to a test solution, said apparatus comprises:

holding means for holding a test solution containing a primer, substrate molecules at least one of which is labeled with a marker molecule capable of generating a detectable signal, a nucleic acid synthase, and a target nucleic acid;

measuring means for measuring a signal from the marker molecule after initiation of a nucleic acid amplification reaction in the test solution;

evaluation means for evaluating mobility of the marker molecule in the test solution on the basis of the signal detected; and

data output means for outputting a evaluation result obtained by the evaluation means as a quantification data of the target nucleic acid molecule.

**Claim 10. (withdrawn)** An apparatus according to claim 9, wherein the measuring means comprises an optical system for performing measurement in a micro detection field brought within a diffraction-limited region near a focal point.

**Claim 11. (withdrawn)** An apparatus according to claim 9, wherein the measuring means comprises a microscope for performing

measurement in a micro field of vision formed by a confocal optical system.

**Claim 12. (withdrawn)** An apparatus according to claim 10 or 11, wherein the diffraction-limited region is formed of an aperture having an average diameter of  $30 \pm 20 \mu\text{m}$ .

**Claim 13. (withdrawn)** An apparatus according to claim 10 or 11, wherein the diffraction-limited region is formed of an aperture having an average diameter of  $20 \pm 10 \mu\text{m}$ .

**Claim 14. (withdrawn)** An apparatus according to claim 10 or claim 11, wherein the micro detection field is a virtually a cylindrical region having an average radius of  $200 \pm 50 \text{ nm}$  and an average length on an optical axis of  $2000 \pm 500 \text{ nm}$ .

**Claim 15. (withdrawn)** An apparatus according to claim 9, wherein the evaluation means comprises a means for storing a plurality of measurement data obtained in a predetermined time,

and an arithmetically operating means for processing the plurality of measurement data stored in accordance with an autocorrelation function.

**Claim 16. (withdrawn)** An apparatus according to claim 15, wherein the evaluation means comprises a means for storing measurement data regarding to a plurality of marker molecules obtained in a measurement area, and an arithmetic operating means for processing the measurement data stored per each of the marker molecules in accordance with the autocorrelation function.

**Claim 17. (withdrawn)** An apparatus according to claim 15 or 16, wherein the data output means includes a conversion means for converting the measurement data into statistical data expressing a positional change with time with respect to a plurality of monitoring data.

**Claim 18. (withdrawn)** A method of quantitatively analyzing a target nucleic acid molecule present in a biological sample,

comprising:

an amplifying step of amplifying the target nucleic acid by using first and second primer molecules having sequences which are complementary with two discrete nucleotide sequence regions of the target nucleic acid molecule respectively, at least one of the first and second primers being labeled with a detectable marker molecule and at least the number of labeled primer molecules being known;

a measurement step of obtaining measurement data regarding the labeled molecule by using at least a part of a test solution which has been subjected at least a single amplification step; and

a determination step of determining a number and a size of the target nucleic acid molecules on the basis of the measurement data.

**Claim 19. (withdrawn)** A method according to claim 18, wherein the amplification step is performed by using the first and second primers which are contained in a mix ratio selected to attain asymmetric nucleic acid amplification.

**Claim 20. (withdrawn)** A method according to claim 18, wherein the number of one of the first and second primers is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule.

**Claim 21. (withdrawn)** A method according to claim 20, wherein the mix ratio of the first and second primers in the test solution falls within a range of 2:1 to 20:1.

**Claim 22. (withdrawn)** A method according to claim 20, wherein a mix concentration ratio of the first and second primers in the test solution falls within a range of 800 nM :400 nM to 800 nM :40 nM.

**Claim 23. (withdrawn)** A method according to claim 18, wherein the measurement step includes

- a step of obtaining a plurality of measurement data within a predetermined time interval, in a micro detection field capable of identifying individual marker molecules;
- a step of converting the plurality of measurement data into a statistical data showing a positional change with time; and



a step of determining a number of target nucleic acid molecules on the basis of the statistical data.

**Claim 24. (withdrawn)** A method according to claim 23, further comprising a step of performing arithmetic operation of the statistical data by use of autocorrelation function.

**Claim 25. (withdrawn)** A method according to claim 24, wherein a fluctuation motion of the marker molecule in the test solution is measured in the measurement step.

**Claim 26. (withdrawn)** A method according to any one of claims 18 to 25, wherein the determination step is performed on the basis of a curve which is obtained by plotting the statistical data and shows a dynamic change of the target molecule.

**Claim 27. (withdrawn)** A method according to claim 23, wherein the measurement step is performed in a three dimensional micro detection field.

**Claim 28. (withdrawn)** A method according to claim 27, wherein the micro detection field in the measurement step is formed by a confocal optical system.

**Claim 29. (withdrawn)** A method according to claim 28, wherein the micro detection field is a diffraction-limited region near a focal point.

**Claim 30. (withdrawn)** A method according to claim 29, wherein the diffraction-limited region is formed by a pin hole having an average diameter of  $30 \pm 20 \mu\text{m}$ .

**Claim 31. (withdrawn)** A method according to claim 29, wherein the diffraction-limited region is formed by a pin hole having an average diameter of  $20 \pm 10 \mu\text{m}$ .

**Claim 32. (withdrawn)** A method according to any one of claims 27 to 31, wherein the micro detection field is virtually a cylindrical region having an average radius of  $200 \pm 50 \text{ nm}$  and an average length on an optical axis of  $2000 \pm 500 \text{ nm}$ .

**Claim 33. (withdrawn)** A method according to claim 18, wherein the marker molecule comprises a fluorescent dye.

**Claim 34. (withdrawn)** A method according to claim 33, wherein the fluorescent dye generates a detectable signal both before and after hybridization.

**Claim 35. (withdrawn)** A method according to claim 34, wherein the fluorescent dye is selected from the group consisting of FITC, DAPI, rhodamine, Cy 3, Cy 3.5, Cy 5, Cy 5.5, and Cy 7.

**Claim 36. (withdrawn)** A method according to claim 32 or 33, wherein the measurement step is performed by a measurement mode for measuring a single photon.

**Claim 37. (withdrawn)** A method according to claim 18, wherein the 10 amplification step is performed in a number of cycles which is determined depending upon an amount of the target nucleic acid molecule.

**Claim 38. (withdrawn)** A method according to claim 26, wherein the micro detection field is virtually a cylindrical

region having an average radius of  $200 \pm 50$  nm and an average length of an optical axis of  $2000 \pm 500$  nm.

**Claim 39. (previously presented)** A method according to any one of claims 1 to 5, wherein the number of labeled primer molecules is known.

**Claim 40. (canceled)**

**Claim 41. (canceled)**

**Claim 42. (previously presented)** A method according to claim 1, wherein the mixing ratio of the forward primer and the reverse primer is in a range of 2:1 to 20:1.

**Claim 43. (previously presented)** A method according to claim 42, wherein the mixing ratio of the forward primer and the reverse primer is in a range of 800nM : 400nM to 800nM : 40nM.